albumin, wherein the PQQ-dependent glucose dehydrogenase content is 100 to 2000 kU per gram of the total components calculated on a dry basis.

a2

4. (Amended) The method according to claim 3, wherein the PQQ-dependent glucose dehydrogenase is present in the composition with a buffer.

add B2

REMARKS

The Present Invention

The present invention relates to a stable composition comprising a PQQ-dependent glucose dehydrogenase and a method of preparing the composition.

The Pending Claims

Claims 1-4 are currently pending. Reconsideration of the pending claims is respectfully requested.

Amendments to the Claims

The claims have been amended so as to more particularly point out and distinctly claim the invention. In particular, the claims have been amended to correct grammar and to recite that the PQQ-dependent glucose dehydrogenase content is 100 to 2000 kU per gram of the composition. This amendment is supported by the specification at, for example, page 6, lines 2-3. No new matter has been added by way of these amendments. The precise amendments to the claims, as well as the text of the pending claims as amended, are set forth on separate attachments hereto.

Summary of the Office Action

The Office Action rejects claims 3 and 4 under 35 U.S.C. § 112, second paragraph, as allegedly indefinite. Claims 3 and 4 are rejected under 35 U.S.C. § 102(b) as anticipated by Adachi et al. (JP 09-140378). Claims 1-4 are rejected under 35 U.S.C. § 103(a) as obvious over Sode et al. (*Biotechnology Techniques*, *11*(8), 577-580 (1997)) in view of Adachi et al. (JP 09-140378).

Discussion of the Section 112, Second Paragraph, Rejection

Claims 3 and 4 are rejected under section 112, second paragraph, because the Examiner considers the phrase "is made to coexist" vague and indefinite. Claims 3 and 4

have been amended to correct the grammar of these claims. The section 112 rejection should be withdrawn in view of the amended claims.

Discussion of the Anticipation Rejection

Adachi et al. discloses a PQQ-dependent glucose dehydrogenase composition comprising (1) a calcium ion or calcium salt, (2) at least one amino acid selected from the group consisting of glutamic acid, glutamine, and lysine, and (3) serum albumin. Adachi et al. teaches that the composition can be either aqueous or lyophilized, as long as it comprises the above-described amino acid(s) as a stabilizing agent (paragraph [0014]).

Adachi et al. does not specifically teach a range of the glucose dehydrogenase concentration, and hence does not teach its activity, in the composition. However, Example 4 describes an aqueous composition comprising 5 U/ml (5000 U/L) of PQQ-dependent glucose dehydrogenase, 29.5 mM of 1-methoxy-5-phenazolium methylsulfate, 0.6 mM of MTT, 0.2 mM of NaN_3, 10 mM of $CaCl_2$, 0.05% of glutamine, 0.05% of glutamic acid, 0.05% of lysine and 0.2% of bovine serum albumin in 50 mM PIPES buffer (pH 7.5). On a weight basis, therefore, one liter of the composition contains 9.9 g of 1-methoxy-5-phenazolium methylsulfate (MW 336.36), 249 mg of MTT (MW 414.32), 13 mg of NaN_3 (MW 65.01), 1.1 mg of $CaCl_2$ (MW 110.98), 0.5 g of glutamine, 0.5 g of glutamic acid, 0.5 g of lysine, 2 g of bovine serum albumin, and 15.1 g of PIPES buffer (MW 302.37), which represents approximately 28.5 g of these components in total. Therefore, the glucose dehydrogenase activity relative to the total dry weight in the aqueous composition is 5000 U/28.5 g or 0.18 kU/g.

In contrast, the composition used in the methods of pending claims 3 and 4 has an enzyme activity of 100 to 2000 kU glucose dehydrogenase/g, which is remarkably higher than that of Adachi et al. -- at least 556 times higher. Accordingly, Adachi et al. does not teach the composition of the method according to claims 3 and 4, and Adachi et al. does not anticipate the present invention.

Furthermore, Adachi et al. does not render the present invention, as defined by claims 3 and 4, obvious. Adachi et al. does not teach or suggest a method involving the use of a composition comprising a PQQ-dependent glucose dehydrogenase with any specified activity, let alone a composition with an enzyme activity that is 100 to 2000 kU per gram of the total components calculated on a dry basis. Nor, for that matter, does Adachi et al. teach or suggest the benefits of having a composition with the recited amount of PQQ-dependent glucose dehydrogenase.

In the context of the present invention, an enzyme composition, in particular a lyophilized enzyme composition, ultimately is used in the form of an aqueous solution as

an enzyme reagent. If the lyophilized composition has low enzyme activity, a large amount of the composition is necessary for preparing the enzyme solution. Consequently, the solution would have a high viscosity due to the high concentration of stabilizing agent present (e.g., aspartic acid, glutamic acid, α-ketoglutaric acid, malic acid, α-ketogluconic acid, α-cyclodextrin, and their salts). The increased concentration of the stabilizing agent can cause an error in measuring a small amount of the solution with a pipet. In addition, the solution would contain increased amounts of impurities that can adversely affect the enzyme reaction.

Since the composition according to the present invention requires a much higher enzyme activity relative to the total solid weight than that of Adachi et al., a very small amount of lyophilized composition is needed to prepare a solution with the desired enzyme activity. This advantage allows measurement errors and side effects by impurities to be greatly reduced due to a low concentration of stabilizing agent in the solution. Adachi et al. is completely silent on these problems and does not teach or suggest ways of modifying the composition in order to address such issues.

Since Adachi et al. fails to teach or suggest an enzyme activity of 100-2000 kU/g in the composition and corresponding method, the present invention as defined by claims 3 and 4 is novel and unobvious. The section 102 rejection should be withdrawn.

Discussion of the Obviousness Rejection

As discussed above, Adachi et al. does not teach or suggest a composition or method in which the PQQ-dependent glucose dehydrogenase content is 100 to 2000 kU per gram of the total components calculated on a dry basis.

Sode et al. does not cure the deficiencies of Adachi et al. Sode et al. discloses that a lyophilized mixture of PQQ-dependent glucose dehydrogenase and an additive such as trehalose, betaine, or ammonium sulfate, shows a smaller decrease in enzyme activity after 1 hour of incubation at 30-80 °C than that of a lyophilized enzyme alone (Figure 2). The enzyme composition taught by Sode et al. does not have a PQQ-dependent glucose dehydrogenase activity of 100 to 2000 kU per gram of the total components calculated on a dry basis nor does the composition contain the stabilizing compounds recited in pending claims 1-4. Accordingly, Sode et al. fails to teach or suggest the composition and method according to the present invention.

As a result, the combination of the disclosures of Adachi et al. and Sode et al. does not result in the present invention as defined by the pending claims. Since neither Adachi et al. nor Sode et al., either alone or in combination, teaches all the elements of

claims 1-4, the present invention is not obvious over these references. The section 103 rejection should be withdrawn.

Conclusion

The application is considered in good and proper form for allowance, and the Examiner is respectfully requested to pass this application to issue. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,

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Date: July 30, 2002

CERTIFICATE OF MAILING

I hereby certify that this RESPONSE TO OFFICE ACTION (along with any documents referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, Washington, D.C. 20231.

Date: July 30, 2002